

**CLAIMS:**

1. A DNA vaccine for treating a T cell-mediated inflammatory autoimmune disease comprising a recombinant construct comprising a nucleic acid sequence encoding a fragment of heat shock protein 60 (HSP60) characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.
2. The DNA vaccine of claim 1, wherein the fragment is derived from human HSP60.
3. The DNA vaccine of claim 2, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids human 496-515 of HSP60 (SEQ ID NO:9).
4. The DNA vaccine of claim 1, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences in a suitable expression system enabling *in vivo* expression of the encoded fragment in a human host.
5. The DNA vaccine of claim 4, wherein the transcription control sequences are selected from the group consisting of: RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.
6. The DNA vaccine of claim 1, wherein the recombinant construct is incorporated into an eukaryotic expression vector.
7. The DNA vaccine of claim 6, wherein the eukaryotic expression vector is selected from the group consisting of: pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV and pTRES.
8. A recombinant construct comprising a nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal

vaccinated with HSP70 to induce Th2/3 T-cell responses, the nucleic acid sequence being operatively linked to one or more transcription control sequences.

9. The construct of claim 8, wherein the fragment is derived from human HSP60.

10. The construct of claim 9, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

11. The construct of claim 8, wherein the transcription control sequences are selected from the group consisting of: RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.

12. The construct of claim 8, wherein the recombinant construct is incorporated into an eukaryotic expression vector.

13. The construct of claim 12, wherein the eukaryotic expression vector is selected from the group consisting of: pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV and pTRES.

14. A pharmaceutical composition comprising (a) a recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses; and (b) a pharmaceutically acceptable carrier.

15. The composition of claim 14, wherein the carrier comprises a delivery vehicle that delivers the nucleic acid sequences to a subject.

16. The composition of claim 15, wherein the delivery vehicle is selected from the group consisting of liposomes, micelles, emulsions and cells.

17. The composition of claim 14, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences

18. The composition of claim 17, wherein the transcription control sequences are selected from the group consisting of: RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.
- 5 19. The composition of claim 14, wherein the recombinant construct is incorporated into an eukaryotic expression vector.
20. The composition of claim 19, wherein the eukaryotic expression vector is selected from the group consisting of: pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV and pTRES.
- 10 21. The composition of claim 14, wherein the fragment is derived from human HSP60.
22. The composition of claim 21, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).
- 15 23. A pharmaceutical composition comprising (a) a peptide fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses; and (b) a pharmaceutically acceptable carrier.
- 20 24. The composition of claim 23, wherein the fragment is derived from human HSP60.
- 25 25. The composition of claim 23, wherein the fragment comprises amino acid sequence selected from: amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), and amino acids 496-515 of human HSP60 (SEQ ID NO:9).
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26. A method of treating a T cell-mediated inflammatory autoimmune disease, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct, the recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.
27. The method of claim 26, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.
28. The method of claim 26, wherein the fragment is derived from human HSP60.
29. The method of claim 28, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).
30. The method of claim 26, wherein the T cell-mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.
31. The method of claim 26, wherein the subject is selected from the group consisting of humans and non-human mammals.
32. The method of claim 26, wherein the pharmaceutical composition is administered to said subject at the time of appearance of disease symptoms.
33. The method of claim 26, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.
34. A method of preventing the symptoms of a T cell-mediated inflammatory autoimmune disease, comprising administering to a subject in need thereof a

prophylactically effective amount of a pharmaceutical composition comprising a recombinant construct, the recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.

35. The method of claim 34, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.

36. The method of claim 34, wherein the fragment is derived from human HSP60.

37. The method of claim 36, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

38. The method of claim 34, wherein the T cell-mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.

39. The method of claim 34, wherein the subject is selected from the group consisting of humans and non-human mammals.

40. The method of claim 34, wherein the pharmaceutical composition is administered to said subject prior to the appearance of disease symptoms.

41. The method of claim 34, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.

42. A method for treating a T cell-mediated inflammatory autoimmune disease comprising the steps of (a) obtaining cells from a subject; (b) transfecting the cells *in vitro* with a recombinant construct comprising an isolated nucleic acid sequence

encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses; and (c) reintroducing the transfected cells to the subject, thereby treating the disease.

43. The method of claim 42, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.

44. The method of claim 42, wherein the transfected cells are administered to said subject at the time of appearance of disease symptoms.

45. The method of claim 42, wherein the T-cell mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.

46. The method of claim 42, wherein the subject is selected from the group consisting of humans and non-human mammals.

47. The method of claim 42, wherein the fragment is derived from human HSP60.

48. The method of claim 47, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

49. A method for preventing the symptoms of a T cell-mediated inflammatory autoimmune disease comprising the steps of (a) obtaining cells from a subject; (b) transfecting the cells *in vitro* with a recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses; and (c) reintroducing the transfected cells to the subject, thereby preventing the symptoms of the disease.

50. The method of claim 49, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.
51. The method of claim 49, wherein the transfected cells are administered to said subject prior to the appearance of disease symptoms.
- 5 52. The method of claim 49, wherein the T-cell mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.
- 10 53. The method of claim 49, wherein the subject is selected from the group consisting of humans and non-human mammals.
54. The method of claim 49, wherein the fragment is derived from human HSP60.
55. The method of claim 54, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).
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56. A method of treating arthritis, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct, the recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.
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57. The method of claim 56, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.
58. The method of claim 56, wherein the fragment is derived from human HSP60.
- 30 59. The method of claim 58, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino

acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

60. The method of claim 56, wherein the pharmaceutical composition is administered to said subject at the time of appearance of disease symptoms.

61. The method of claim 56, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.

62. A method of preventing the symptoms of arthritis, comprising administering to a subject in need thereof a prophylactically effective amount of pharmaceutical composition comprising a recombinant construct, the recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.

63. The method of claim 62, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.

64. The method of claim 62, wherein the fragment is derived from human HSP60.

65. The method of claim 64, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

66. The method of claim 62, wherein the pharmaceutical composition is administered to said subject prior to the appearance of disease symptoms.



67. The method of claim 62, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.
- 5 68. A method for treating or preventing a T cell-mediated inflammatory autoimmune disease comprising the steps of (a) obtaining cells from a subject; (b) infecting the cells *in vitro* with a virus comprising a recombinant construct comprising an isolated nucleic acid sequence encoding a fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses; and (c) reintroducing the infected cells to the subject,
- 10 thereby treating the disease.
69. the method of claim 68, wherein the nucleic acid sequence is operatively linked to one or more transcription control sequences.
70. The method of claim 68, wherein the infected cells are administered to said subject at the time of appearance of disease symptoms.
- 15 71. The method of claim 68, wherein the T-cell mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.
- 20 72. The method of claim 68, wherein the subject is selected from the group consisting of humans and non-human mammals.
73. The method of claim 68, wherein the fragment is derived from human HSP60.
74. The method of claim 73, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino
- 25 acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids
- 30 496-515 of human HSP60 (SEQ ID NO:9).

- 5 75. A method of treating or preventing a T cell-mediated inflammatory autoimmune disease, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a peptide fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.
76. The method of claim 75, wherein the fragment is derived from human HSP60.
- 10 77. The method of claim 76, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).
- 15 78. The method of claim 75, wherein the T cell-mediated inflammatory autoimmune disease is rheumatoid arthritis, collagen II arthritis, multiple sclerosis, autoimmune neuritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, sarcoidosis, autoimmune uveitis, inflammatory bowel disease (Crohn's and ulcerative colitis) or autoimmune hepatitis.
- 20 79. The method of claim 75, wherein the subject is selected from the group consisting of humans and non-human mammals.
80. The method of claim 75, wherein the pharmaceutical composition is administered to said subject at the time of appearance of disease symptoms.
- 25 81. The method of claim 75, wherein the pharmaceutical composition is administered to said subject prior to the appearance of disease symptoms.
82. The method of claim 75, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.
- 30 83. A method of treating or preventing arthritis, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition

comprising a peptide fragment of HSP60 characterized in that it reacts with T cells isolated from an animal vaccinated with HSP70 to induce Th2/3 T-cell responses.

84. The method of claim 83, wherein the fragment is derived from human HSP60.

85. The method of claim 84, wherein the fragment comprises amino acid sequence selected from: amino acids 271-290 of human HSP60 (SEQ ID NO:1), amino acids 346-365 of human HSP60 (SEQ ID NO:2), amino acids 361-380 of human HSP60 (SEQ ID NO:3), amino acids 391-410 of human HSP60 (SEQ ID NO:4), amino acids 406-425 of human HSP60 (SEQ ID NO:5), amino acids 436-455 of human HSP60 (SEQ ID NO:6), amino acids 466-485 of human HSP60 (SEQ ID NO:7), amino acids 481-500 of human HSP60 (SEQ ID NO:8) and amino acids 496-515 of human HSP60 (SEQ ID NO:9).

86. The method of claim 83, wherein the subject is selected from the group consisting of humans and non-human mammals.

87. The method of claim 83, wherein the pharmaceutical composition is administered to said subject at the time of appearance of disease symptoms.

88. The method of claim 83, wherein the pharmaceutical composition is administered to said subject prior to the appearance of disease symptoms.

89. The method of claim 83, wherein the pharmaceutical composition is administered by intravenous injection, intramuscular injection, aerosol, oral, percutaneous or topical administration.

90. A method of screening for active fragments of HSP60 capable of inducing Th2/3 T-cell responses comprising:

(a) applying a DNA construct encoding HSP70 to an animal in a sufficient amount to induce HSP70 expression in the animal;

(b) obtaining T cells from said animal;

(c) contacting the cells with a candidate HSP60 fragment for sufficient time for inducing cytokine secretion in said cells; and

(d) determining the secretion of IL-10, TGF $\beta$ 1 and IFN $\gamma$  from said cells, wherein if the secretion of IL-10 and TGF $\beta$ 1 is increased and the secretion of IFN $\gamma$  is

decreased than the candidate HSP60 fragment is capable of inducing Th2/3 T-cell responses.